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IMMUNOGENICITY AND EFFICACY OF A HEAT-KILLED WHOLE CELL/B SUBUNIT CHOLERA VACCINE

FINAL REPORT

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FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR56.

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I. PROJECT TITLE:

Immunogenicity and Efficacy of a New Formulation of the Heat-Killed Whole Cell/Recombinant B Subunit (WC/rBS) Cholera Vaccine in Healthy U. S. Students Exposed to Enterotoxigenic coli in Mexico (WRAIR Log #381A, MRDC Log # A-5516).

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III. INTRODUCTION

Objectives

1) To confirm the safety of a WC/rBS cholera vaccine in non-immune US adults, 2) to demonstrate serum immunoglobulin type G and fecal secretory immunoglobulin type A to the antigens used when administered in two oral doses, 3) to establish the efficacy of the WC/rBS vaccine against naturally occurring enterotoxigenic E. coli infection in U.S. adults in Mexico with a comparison between the two study groups of the following endpoints: rates of enterotoxigenic E. coli due to heat-labile (LT) and heat-stable (ST) enterotoxin producing strains during a 5-week period, timing of protection in relationship to vaccine administration, and relationship between enterotoxigenic E. coli diarrhea attack rates and serum IgG as well as fecal IgA antibody responses.

Background

Enterotoxigenic E. coli is the most common cause of travelers' diarrhea throughout the world. When WC/rBS was studied in Bangladesh it conferred 69% protection against LT or LT/ST producing enterotoxigenic E. coli in the first eight months. In another study, 67% protection was noted in the first three months after vaccination and two doses were as effective as three in preventing enterotoxigenic E. coli diarrhea. Recently, the vaccine was given to Finnish travelers to Morocco and found to prevent 60% of enterotoxigenic E. coli disease when compared to a control group. This finding suggests that non-immune subjects also benefit by orally administered immunizing agents and the present study was designed.

Study Design

A. Rationale

The rationale for immunizing U.S. subjects in Mexico was: 1) exploring the practical problems of safely administering vaccine to U.S. subjects in country, and 2) the desire to assess the timing of development of protection after initiation of vaccination. The scientific rationalization for designing a study of vaccination upon arrival in country was previous information during the summers of 1986-1987 showing only 57% of enterotoxigenic E. coli disease occurred during the first 2 weeks after arrival in Mexico (Table 1), leaving a substantial number of cases for analysis of the development of protection, which was hypothesized to occur during the third week.

B. Design Specifics

The overall design of the study can be seen in Table 2. A subset of patients was vaccinated twice in the United States for safety testing. This group also received a third dose of vaccine upon arrival in Mexico. The larger group of patients was vaccinated upon arrival in either Guadalajara, Cuernavaca, or Morelia, Mexico. They received doses again on day 10. All subjects submitted stool samples with every illness that developed in order to keep track of enterotoxigenic E. coli disease.

Inclusion criteria for these subjects included: 1) U.S. civilians, men and women ages 18 or over, 2) willingness to participate in the study, 3) willingness to sign informed consent.

Exclusion criteria included: 1) unable to give adequate follow-up examinations in Mexico, 2) unable to submit a stool or serum specimens, 3) failure to understand the nature and plan of the study, 4) use of oral or parenteral antibiotics in the previous 7 days, 5) use of two doses of anti-diarrheal medications in the previous 7 days, 6) history of gastro-intestinal surgery, colitis, or other chronic lower GI tract illness, 7) significant abnormalities detected by screening of the medical history and physical examination, 8) a positive pregnancy test or presently nursing an infant, 9) allergic reaction to any vaccine (such as hives, angioedema or anaphylaxis).

C. Vaccine Administration

In the fasted state WC/rBS vaccine was administered as 3 ml of vaccine in 150 ml of distilled water to which 3.8 grams of sodium bicarbonate and 1.5 grams of citric acid were added. The placebo buffer solution was identical with the exception that the 3 ml dose of vaccine was not added. Vaccine or placebo was prepared out-of-sight of the subjects and out-of-sight of the clinic personnel who followed the subjects in order to maintain double blinding.

D. Vaccine Accountability

See Table 3 for an accounting of vaccine usage. Nine bottles of vaccine were received. The number of bottles was disbursed to the various sites as indicated in Table 2 in excess of the amount anticipated in order to allow for difficulties in transportation, refrigeration, and wastage and accidents during preparation. Six hundred twenty-six doses were delivered. Excess at the various sites was destroyed on site.

E. Analysis

All data was entered on laptop computers and through a series of communications between the U.S. Army and the investigators in Houston, the data was cleaned and a final data set was agreed upon. All decisions as to study outcome and microbiologic designation of enterotoxigenic E. coli was accomplished prior to breaking the code. A number of analysis approaches were anticipated including a survival analysis to estimate the time at which protection developed: however, so few cases of enterotoxigenic E. coli occurred during the time interval of 7 or more days after the second dose that survival analysis was meaningless. Also, no differences between vaccine and placebo was apparent by survival analysis during the entire study. Instead, a density analysis of a number of cases per person days exposed was accomplished assuming from previous publication that protection would develop approximately 7 days after the second dose. Comparison of geometric mean titers of serum and fecal antibody responses was also anticipated; however, serologic testing is not complete and will need to be forwarded as an Addendum.

F. Results

Table 4 shows the attack rate of all enterotoxigenic E. coli disease by week after student arrival for the summer, 1992. Occurrence of enterotoxigenic E. coli was equivalent at the three locations so the locations are lumped in this analysis. Note that the number of persons at risk declines over time due to the differences in program duration between and within the various sites. Of importance, the number of enterotoxigenic E. coli cases per thousand declined precipitously after the second week in country and it was this occurrence which resulted in too little disease occurring after the putative development

of protection for statistical analysis to be meaningful.

Table 5 indicates the flow of vaccine study enrollment. A number of patients never received a vaccine dose despite signing the consent form. Another group dropped out after receiving only 1 dose. The group who was vaccinated in the United States in the safety study was deleted from the analysis of protection since they had been vaccinated so far in advance of arrival in Mexico. Of the 451 subjects who received two doses of vaccine in Mexico, three failed to hand in an adequate number of diaries for analysis, and 448 formed the population for efficacy study analysis. Table 6 shows the demographic breakdown of the 448 subjects in the efficacy analysis. There were no differences between the vaccine and the placebo group.

Table 7 shows the attack rates of LT/ST and ST/LT enterotoxigenic E. coli disease by week after arrival in the present study. Considerably less disease in each ETEC category occurred in the third and fourth weeks when vaccine protection was proposed to occur. Table 8 is the density efficacy analysis comparing enterotoxigenic E. coli and all diarrhea cases that occurred 7 or more days after the second dose of vaccine. Although the percent protection against enterotoxigenic E. coli LT-producing diarrhea of 57% approximated that reported in previous studies, the numbers are too small for statistical significance. Unlike the previous study where protection against other causes of diarrhea was noted, the 16% protection by vaccine against all diarrhea was not statistically significant.

Table 9 shows the comparison of potential adverse reactions the day following a dose of vaccine or placebo among those persons vaccinated in the United States. There were more loose stools passed in the placebo group, but differences were not significant.

Table 10 shows a comparison of potential adverse effects among those vaccinated only in Mexico. In this analysis, with much larger numbers, there was no difference in passage of loose stools or other symptoms following vaccine or placebo.

IV. CONCLUSIONS

Of the total enterotoxigenic E. coli disease that occurred among U.S. students in the four-week period after arrival in Mexico, 57% occurred in the first 2 weeks in 1986 and 1987, but 75% occurred in the first 2 weeks in the present study in 1992. This relatively early onset of enterotoxigenic E. coli disease occurring in 1992 prevented the reliable statistical assessment of WC/rBS cholera vaccine, when this vaccine was hypothesized to become protective 7 days or more after a second dose, i.e. during week three of the study. The oral vaccine was free of adverse reactions compared to placebo and was generally quite well tolerated. What gastro-intestinal symptoms occurred in both groups was probably due to the bicarbonate buffer.

Finally, because enterotoxigenic E. coli disease can occur both substantially and predominately shortly after arrival in a developing country, subjects ideally should be vaccinated with the present vaccine before arrival in the developing country. The first dose of vaccine ideally should be given approximately 3 weeks prior to arrival.

Table 1
Comparison of Enterotoxigenic E. coli Disease by Week after Arriving in the Summer of 1986-1987 and in the Recent (1992) Study

	Total No. ETEC Cases/4 weeks	Percent of ETEC Cases by Week				
		1	2	3	4	3 & 4
1986-87	44	36	21	25	18	43
1992 (present study)	64	36	39	11	14	25

Table 2
Overview of Study Design

	U. S.		Mexico						
Subject Group	1	1	1	2	1	2	2 (10-20%)	2 (80-90%)	1&2
	Week -10	Week -8	Day 0	Day 0	Day 7-10	Day 10-14	Day 15-19	Day 24-28	Day 35-40
Interview, sign consent form, blood screen	X			X					
Health status	X	X	X	X		X			X
Monitoring of side effects (72 hours)	X	X	X	X		X			
Blood drawn for antibody	X			X	X			X	X
Paired stool and blood samples for antibody				X			X		
Vaccine ingestion	X	X	X	X		X			

Table 3
Vaccine Accountability

9 bottles vaccine received	900 doses
No. receiving one dose	289
No. receiving 2nd dose	248
Doses received by those vaccinated in U.S.	89
Doses delivered	626
Wastage	274 doses

Due to logistical constraints, 3 bottles were sent to Cuernavaca, 4 to Guadalajara and 1 to Morelia, and 1 was used to vaccinate students in San Diego CA and Tucson AZ. At each location, remaining vaccine was destroyed on-site due to lack of long term refrigeration.

Table 4
Attack Rate of Total ETEC Disease
by Week after Arrival in 1992

Week	1	2	3	4	5	6
No. at Risk	448	445	412	313	172	47
ETEC cases/1000	51	56	17	29	23	21

Table 5
Vaccine Study Enrollment

Total Enrolled	604
Never received vaccine dose	26
Received at least one dose	578
Dropped out after one dose	82
Received at least two doses	496
Vaccinated in U.S. (safety study)*	45
Received 2 doses in Mexico	451
Received 2 doses in Mexico with adequate follow-up	448 (223 Placebo) (225 Vaccine)

* The number vaccinated in U.S. is higher than 45. Many received only one or two doses and some failed to return to clinic.

Table 6
Demographic Data

	Placebo	Vaccine
Total	223	225
% Females*	63	66
Av. age (yrs)	30	30
Race (%)		
White	86	86
Black	2	1
Hispanic	10	11
Other	2	2
Enrollment sites (%)		
Guadalajara	51	51
Morelia	11	10
Cuernavaca	38	39

* All females were negative for pregnancy.

Table 7
Attack Rate of LT, ST and ST/LT
ETEC Disease by Week
after Arrival in 1992

Week	1	2	3	4
No. at Risk	448	445	412	313
ETEC Cases/1000				
LT	16	18	5	13
ST	16	29	5	10
ST/LT	20	9	7	6

Table 8
Efficacy Analysis: Comparison of ETEC and II Diarrhea Cases
Occurring ≥ 7 Days after Second Dose of Vaccine

	Placebo	Vaccine	% Protection
LT-ETEC			
No. episodes	4	2	
Person Days	1907	2132	
Cases/1000 person days	2.1	0.9	57 (NS)
ST/LT-ETEC			
No. episodes	4	3	
Person Days	1907	2132	
Cases/1000 person days	2.1	1.4	33 (NS)
All Diarrhea			
No. episodes	61	58	
Person Days	1907	2132	
Cases/1000 person days	32	27	16 (NS)

Table 9
Comparison of Potential Adverse Reactions to Vaccine
the Day Following a Dose among Subjects Vaccinated in the U.S.

	Placebo	Vaccine
Total Doses	93	89
Episodes of:		
Loose stools/24h		
1 stool :	10	3
2 stools:	5	4
3 stools:	1	2
any loose stools (%)	18 (19)	10 (11)
Cramps		
mild	3	4
moderate	1	1
severe	0	1
any cramps (%)	4 (4)	6 (7)
Nausea		
mild	1	1
moderate	0	0
severe	0	0
any nausea (%)	1 (1)	1 (1)
Headache		
mild	6	2
moderate	1	2
severe	0	1
any headache (%)	7 (7.5)	5 (5.5)

Table 10
Comparison of Potential Adverse Reactions to Vaccine
the Day Following a Dose

	Placebo	Vaccine
Total Doses	446	450
Episodes of:		
Loose stools/24h		
1 stool :	42	44
2 stools:	22	16
3 stools:	4	5
any loose stools (%)	68 (15)	65 (14)
Cramps		
mild	30	35
moderate	12	11
severe	2	2
any cramps (%)	44 (10)	48 (11)
Nausea		
mild	16	16
moderate	6	5
severe	2	0
any nausea (%)	24 (5)	21 (5)
Headache		
mild	19	30
moderate	11	9
severe	7	4
any headache (%)	37 (8)	43 (9.5)